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Protein

Genome

Structure

PopSet

Taxonomy

OMIM

Bc

Search

PubMed



for



Limits

Preview/Index

History

Clipboard

Details

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Display

Abstract



Sort



Save

Text

Clip Add

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Text Version

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Batch Citation Matcher

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☐ 1: Blood 1994 Jun 1;83(11):3279-88

Related Articles, Links

Idiotypic vaccination against human B-cell lymphoma. Rescue of variable region gene sequences from biopsy material for assembly as single-chain Fv personal vaccines.

Hawkins RE, Zhu D, Ovecká M, Winter G, Hamblin TJ, Long A, Stevenson FK.

Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

Idiotypic determinants on neoplastic B cells could provide tumor antigens for vaccination of patients with B-cell tumors. Because this approach requires an individual vaccine for each patient, simple methods for obtaining idiotypic antigen are desirable. Using polymerase chain reaction (PCR) with family-based V-gene and J-region primers, the variable region genes of heavy and light chains (VH and VL) of Ig have been obtained from biopsy material from 13 patients with B-cell tumors. In each case, analysis of random clones derived from the PCR product showed repeated, clonally-related sequences, whereas normal lymphoid tissue generated no repeated sequences. In 3/3 cases, the repeated sequences were found to be the same as those in a tumor-derived hybridoma. Mutational patterns in the V-genes differed among the tumors, with follicular lymphoma tending to be more highly mutated. The individual VH and VL sequences have been assembled with a flexible linker sequence to encode single-chain Fv (scFv). The scFv sequences can be cloned into bacterial expression vectors to produce protein, or into vectors suitable for direct vaccination using naked DNA. In a model system, expressed scFv protein retained all idiotypic determinants defined by a panel of five anti-idiotypic monoclonal antibodies (MoAbs). Similarly, expressed scFv proteins from two patients were shown to react with anti-idiotypic antibodies. This approach allows production of potential vaccines from surgical biopsies within 2 to 3 weeks.

PMID: 8193363 [PubMed - indexed for MEDLINE]

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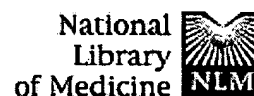


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Nucleotide

Protein

Genome

Structure

PopSet

Taxonomy

OMIM

Bc

Search 

for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

About Entrez

Display

Abstract



Sort



Save

Text

Clip Add

Order

Text Version

Entrez PubMed

Overview

Help | FAQ

Tutorial

New/Noteworthy

E-Utilities

PubMed Services

Journals Database

MeSH Browser

Single Citation Matcher

Batch Citation Matcher

Clinical Queries

LinkOut

Cubby

Related Resources

Order Documents

NLM Gateway

TOXNET

Consumer Health

Clinical Alerts

ClinicalTrials.gov

PubMed Central

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☐ 1: J Immunol 1997 Aug 15;159(4):1885-92

Related Articles, Links

DNA vaccines against lymphoma: promotion of anti-idiotypic antibody responses induced by single chain Fv genes by fusion to tetanus toxin fragment C.

Spellerberg MB, Zhu D, Thompsett A, King CA, Hamblin TJ, Stevenson FK.

Tenovus Laboratory, Southampton University Hospitals, United Kingdom.

Idiotypic determinants can act as tumor-associated Ags for B cell lymphoma. Vaccination with idiotype protein and adjuvant is known to induce specific protection against lymphoma challenge in mice, largely mediated by anti-idiotypic Ab. For facilitating the approach for patients, the V(H) and V(L) genes used to encode the individual idiotype determinants of each tumor can be obtained by PCR and assembled as single chain Fv (scFv). DNA vaccines containing scFv sequences alone induce low and poorly reproducible levels of anti-idiotypic Ab, likely to be insufficient to suppress tumor in patients. In addition, it may be necessary to break tolerance to Id in tumor bearers. By fusing the gene for fragment C of tetanus toxin to the C terminus of human scFv, we have promoted the anti-scFv Ab response in mice by >50-fold in three of three cases. The induced Abs are mainly against idiotype determinants, and react specifically with patients' tumor cells, indicating optimal folding of the scFv molecule in the fusion protein. For both antigenic components of the DNA vaccine, the IgG subclass distribution showed a relative increase in IgG2a as compared with vaccination with IgM protein in adjuvant. In patients, the fusion gene should both promote anti-idiotypic Ab and induce Abs against fragment C of tetanus toxin. The latter response would provide a potentially useful comparative measure of the ability of patients to respond to conventional Ag delivered via DNA.

PMID: 9257853 [PubMed - indexed for MEDLINE]

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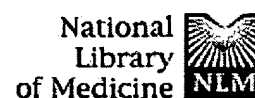


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Nucleotide

Protein

Genome

Structure

PopSet

Taxonomy

OMIM

Bc

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for

Go

Clear

Limits

Preview/Index

History

Clipboard

Details

About Entrez

Display

Abstract



Sort



Save

Text

Clip Add

Order

Text Version

Entrez PubMed

Overview

Help | FAQ

Tutorial

New/Noteworthy

E-Utilities

PubMed Services

Journals Database

MeSH Browser

Single Citation Matcher

Batch Citation Matcher

Clinical Queries

LinkOut

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NLM Gateway

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Clinical Alerts

ClinicalTrials.gov

PubMed Central

Privacy Policy

☐ 1: Plant Mol Biol 1993 Nov;23(4):861-70

Related Articles, Links

Erratum in:

- Plant Mol Biol 1994 Mar;24(5):833

Secretion of a functional single-chain Fv protein in transgenic tobacco plants and cell suspension cultures.

Firek S, Draper J, Owen MR, Gandecha A, Cockburn B, Whitlam GC.

Department of Botany, University of Leicester, UK.

A synthetic gene encoding an anti-phytochrome single-chain Fv (scFv) antibody bearing an N-terminal signal peptide has been used to transform tobacco plants. Immunoblot analysis showed that transformed plants accumulate high levels of scFv protein, accounting for up to 0.5% of the total soluble protein fraction, which could be extracted by simple infiltration and centrifugation of leaf tissue. A substantial proportion of the scFv protein extracted in this way was found to possess antigen-binding activity. Callus cell suspension cultures derived from transformed plants secrete functional scFv protein into the surrounding medium. Compared with the levels of scFv protein observed in plants expressing the native scFv gene, the incorporation of an N-terminal signal peptide, to target the scFv to the apoplast, results in elevated accumulation of the protein.

PMID: 8251638 [PubMed - indexed for MEDLINE]

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